

## Dispersal of invasive and native brine shrimps *Artemia* (Anostraca) via waterbirds

**Abstract**—North American brine shrimp *Artemia franciscana* have been exported worldwide since the 1950s for use in aquarium trade and fish farming. Aquaculture is expanding along the Mediterranean coast, leading to the release of *A. franciscana* into native *Artemia* populations. *A. franciscana* was first detected in 1981 in Portugal and has since spread to saltworks along the East Atlantic flyway used by shorebirds. Once *A. franciscana* becomes established in a locality, native *Artemia* tend to disappear. To test whether migratory shorebirds can disperse invasive and native *Artemia* between wetlands, we extracted *Artemia* cysts from feces and pellets collected at Castro Marim (Portugal) and Cadiz Bay (Spain) during southward migration. We found that large numbers of viable eggs of *A. franciscana* and native *Artemia parthenogenetica* were dispersed by Redshank *Tringa totanus*, Black-tailed Godwit *Limosa limosa*, and other shorebirds migrating through the Iberian Peninsula. This is the most extensive field demonstration to date that invertebrates can disperse readily via gut passage through birds.

Invasion by nonnative species is second only to habitat loss as a threat to global biodiversity, has a huge economic impact, and has had its greatest impact in aquatic ecosystems (Ruiz et al. 1999; Mooney and Cleland 2001). Nonnative aquatic invertebrates are typically moved between continents by man, e.g., in the ballast of ships or intentionally for aquaculture or fisheries purposes (Leppäkoski et al. 2002; Bailey et al. 2003). Once established, they are generally assumed to disperse using their own active mechanisms via ocean or river currents or via intraregional boat traffic (Wasson et al. 2001). The role of migratory birds in spreading exotic invertebrates has not been fully assessed despite the importance of understanding the dispersal mechanisms of invasive species so that their spread can be predicted. Some authors reviewing dispersal mechanisms of aquatic invasives have made no mention of a potential role for birds (Leppäkoski et al. 2002).

Darwin attributed much importance to the role of migratory waterbirds as dispersers of invertebrates ("The wide distribution of fresh-water plants and of the lower animals I believe mainly depends on the wide dispersal of their seeds and eggs by animals, more especially by fresh-water birds, which have great powers of flight, and naturally travel from one piece of water to another," Darwin 1859). He suggested that shorebirds have a particularly important role and proposed that alien snails extended their range by dispersing on the feathers or feet of birds (Darwin 1859). Various laboratory studies have since demonstrated that propagules can survive passage through the digestive system (Figuerola and Green 2002a). However, few studies have demonstrated such dispersal in the field (Proctor 1964; Figuerola and Green 2002a).

Owing to similar morphology, until the 1980s, all populations of brine shrimps *Artemia* (Crustacea, Anostraca) were considered strains of a single species, but six sexual species are now recognized, together with a heterogeneous group of parthenogenetic populations, under the binomen *Artemia*

*parthenogenetica* (Abatzopoulos et al. 2002). The sexual *Artemia franciscana* is widespread in the Americas. In the Mediterranean region, the native species are the sexual *Artemia salina* (formerly *Artemia tunisiana*) and the *A. parthenogenetica* (mainly diploid and tetraploid populations, Abatzopoulos et al. 2002). Since the 1950s, *A. franciscana* cysts have been commercially exported worldwide from San Francisco Bay and Great Salt Lake, U.S.A., for use in aquaculture and in the aquarium and pet trade (Abatzopoulos et al. 2002). In the Mediterranean region, many former saltworks in which salt production has become unprofitable are being transformed into aquaculture facilities, leading to the release of *A. franciscana* into hatcheries and its escape into habitats with native *Artemia* populations (Amat et al. 2005). Given its use in the pet trade and in microscope sets, indiscriminate releases into other saline wetlands suitable for *Artemia* are also possible.

Between 1993 and 2003, *Artemia* cysts (resting eggs) from numerous saltworks in the West Mediterranean were collected, and their species composition determined (Amat et al. 2005; authors unpubl.). By then, *A. franciscana* was the only species recorded in Portugal, where it is found in areas both with and without aquaculture. It is also found in Cadiz Bay (Spain), two French sites, and one in Morocco. *A. franciscana* was first detected in 1981 in the Portuguese Algarve (Amat et al. 2005) and has since spread to all the Iberian sites considered to be most important for shorebirds along the East Atlantic Flyway: Tejo, Aveiro, Faro, and Sado in Portugal and Cadiz Bay (Boyd and Pirot 1989; Amat et al. 2005). To date, there has been no spread detected to the many saltworks east of Gibraltar outside the flyway (Fig. 1). At Aveiro, only *A. parthenogenetica* was recorded in 1985, but by 1991, it had been replaced by *A. franciscana*.

In this study, we assessed the ability of shorebirds (the most abundant waterbirds in coastal saltworks) to disperse *A. franciscana* and *A. parthenogenetica* by sampling two areas where *A. franciscana* has been recorded: Castro Marim (Portugal, 37°12'N, 7°26'W) and Cadiz Bay (36°27'N, 6°11'W). Both are situated on the Atlantic coast of the southwestern part of the Iberian Peninsula (Fig. 1) and are listed as wetlands of international importance under the Ramsar Convention (<http://www.wetlands.org/RSDB/default.htm>). Both sites hold saltworks and tens of thousands of shorebirds during migration periods (Hortas 1997).

**Materials and methods**—We collected fresh feces and pellets from roost sites used by monospecific flocks in saltworks at Castro Marim on 23 July 2002 and Cadiz Bay from 22 July 2002 to 3 August 2002. Several shorebirds, including the Redshank *Tringa totanus*, regurgitate pellets after digestion. The dates of sampling coincided with the beginning of the southward (autumn) shorebird migration (Hortas 1997). Each sample of feces or pellet was carefully separated from the soil (discarding that part in contact with soil) and placed

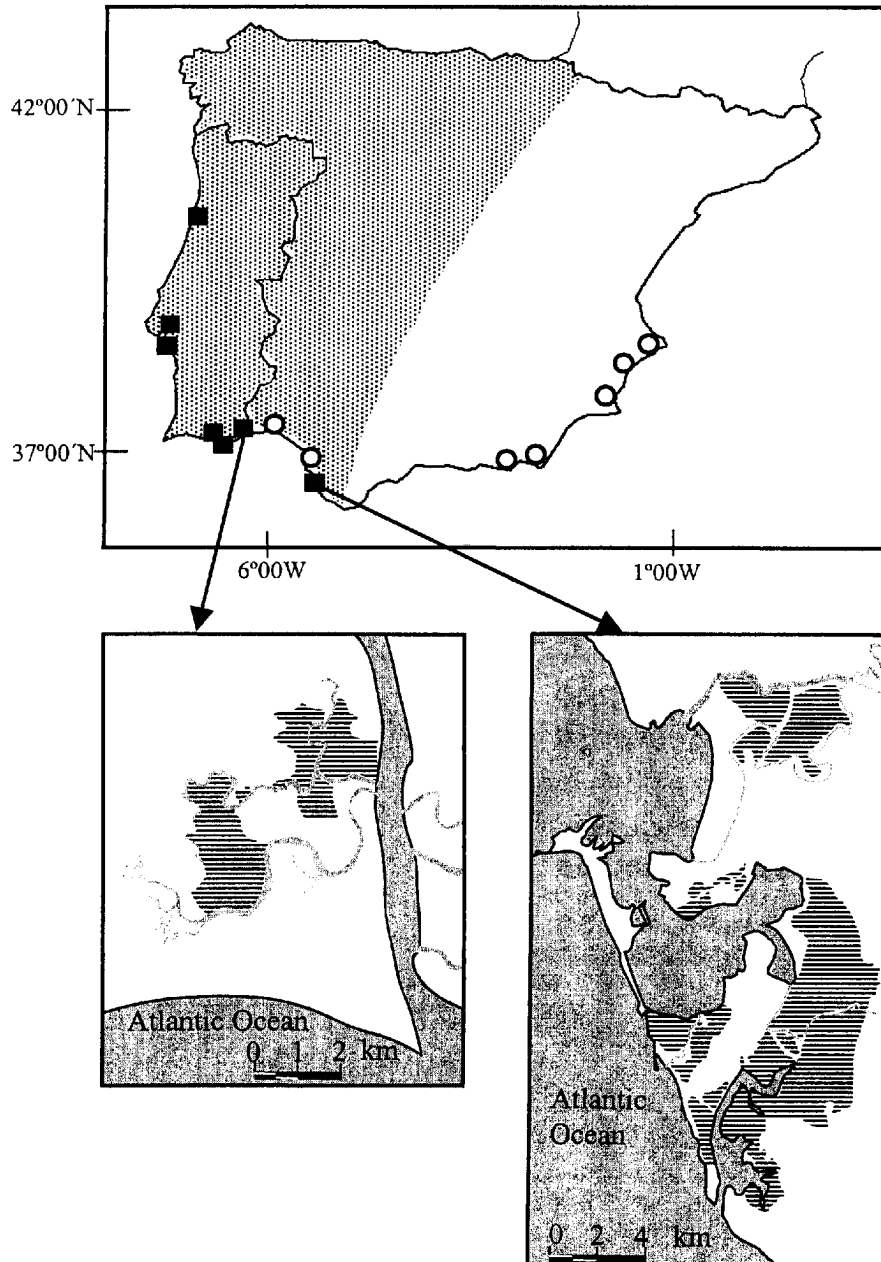


Fig. 1. Castro Marim (left insert) and Cadiz Bay (right) on a map of the Iberian Peninsula, showing the current distribution of *A. franciscana* (black squares) and native *Artemia* (white dots) in relation to the East Atlantic Flyway (shaded after Stroud et al. 2004). Hatched areas within inserts represent saltworks. Other *Artemia* sites with no information on species composition since 1992 are not shown.

in a tube. Given the number of birds present and the freshness of the samples, we are confident that each sample was from a different bird. The samples were stored at 5°C, and within a month, *Artemia* cysts were extracted by washing them in a 0.04-mm sieve and resuspension in hypersaline brine in which intact cysts float. The cysts were counted, washed in distilled water, and then dried for 48 h at 40°C.

The cysts were then incubated in diluted, filtered seawater (25 g L<sup>-1</sup>) at 26°C and under continuous illumination for 48 h. Hatched nauplii were counted, transferred into 60-cm<sup>3</sup> vessels, and cultured in 70 g L<sup>-1</sup> filtered brine (seawater plus crude sea salt) on a diet of live *Dunaliella salina* and *Tetraselmis suecica*. They were maintained at 24°C, under aeration on a 12:12 light:dark (LD) photoperiod. The medium

The birds we studied included a mixture of birds that had just completed or were just about to commence long-distance movements and birds that were making movements between feeding and roosting spots within our study sites. The average time spent by shorebirds at stopover sites during migration is unknown for our study sites, making it impossible to assess what proportion of the cysts extracted from feces or pellets were transported from long distances. However, the poor match between the species composition of *Artemia* cysts extracted and those from adjacent salt pans shows that the birds were transporting cysts between different parts of wetland complexes. Redshank, godwits, and other shorebirds regularly move between salt pans separated by <20 km within stopover sites (Potts pers. comm.), favoring rapid *Artemia* dispersal within and between nearby saltworks. Dominance of *A. parthenogenetica* in bird samples from Cadiz (Table 1) probably reflects feeding in salt pans that had been dry since *A. franciscana* invaded, allowing native *Artemia* to hatch from cysts in sediments upon reflooding (cysts can remain viable for decades in sediments, Abatzopoulos et al. 2002). There are hundreds of pans in Cadiz Bay, and it was not possible to know exactly where the birds had been feeding when we collected feces.

Redshank and godwits fly at 56–60 km h<sup>-1</sup> (Welham 1994). In a laboratory study, the modal retention time (much less than the mean) of viable *A. franciscana* cysts defecated by another shorebird, the Killdeer *Charadrius vociferus* (size intermediate between redshank and godwit), was found to be 90 min, and the maximum was 26 h (Proctor et al. 1967). This suggests that, during migration, the maximum dispersal distance of viable cysts would be about 1,500 km. Studies of gut passage of viable *A. franciscana* cysts in other birds suggests that the maximum retention time increases with body size, ranging from 3 h in a canary *Serinus canarius* to at least 38 h in a Shelduck *Tadorna tadorna* (Proctor 1964; MacDonald 1980).

Radio and satellite tracking shows that shorebirds move rapidly between coastal wetlands, and distances between stopovers can exceed 1,000 km (Iverson et al. 1996). *A. franciscana* cysts are also likely to be transported internally by some of the other 24 migratory shorebird species using the East Atlantic Flyway (Stroud et al. 2004), as well as other birds that consume *Artemia* and move frequently between wetlands. *Artemia* cysts are consumed by Greater Flamingos *Phoenicopterus ruber* and Shelduck in the wild (MacDonald 1980). Viable cysts are also likely to be transported externally on the feathers and feet of waterbirds (Figuerola and Green 2002b). We do not expect that such dispersal processes are restricted to these study sites or to autumn migration. In a study of Redshank diet at the Odiel marshes in Spain (37°17'N, 6°55'W), cysts were similarly abundant in pellets in both spring and autumn migration (Sánchez et al. 2005).

We observed considerable variation among waterbird species and localities in the number of intact *Artemia* cysts transported and in their viability (Tables 1, 2). This variation is likely to be related to differences between bird populations in habitat use, diet, gut morphology, and retention time (Figuerola and Green 2002a). Feces of both Redshank and godwits contained remains of adult *Artemia*, and these birds are likely to ingest cysts that are still inside adult *Artemia* (some

of which may have been immature and thus less viable, see Bohonak and Whiteman 1999) as well as those loose on the water surface or in sediments. The greater viability of Redshank cysts recovered from feces rather than pellets furthers long-distance dispersal because cysts are likely to be retained longer when expelled as feces than when regurgitated (Nogales et al. 2001). Cysts expelled in pellets may be less viable because they are ground together with grit and hard prey items in the gizzard (Sánchez et al. 2005). Adding grit to cysts fed to flamingos reduces their viability after gut passage (MacDonald 1980).

At Castro Marim, the hatching success of *A. franciscana* cysts taken from bird samples was much lower than for cysts collected in adjacent salt pans. Hatching success of cysts from Redshank pellets at Cadiz Bay was higher than for cysts from the closest *A. parthenogenetica* site we could find at Sanlúcar de Barrameda. Such comparisons are difficult to interpret as floating cysts collected at the edge of salt pans may not be the ones ingested by the birds and may have different viabilities. Gut passage through the Greater Flamingo and Shelduck has been shown experimentally to reduce hatching success of *A. parthenogenetica* cysts (MacDonald 1980).

Although there are few data available on the timing of arrival of *A. franciscana* at different locations or on the timing and location of introductions by people, the current distribution of this exotic in the Iberian Peninsula is consistent with expansion via shorebirds. Of nine saltworks studied on the main East Atlantic Flyway west of Gibraltar since 1993 (Amat et al. 2005; authors unpubl.), *A. franciscana* is dominant at seven of them (Fig. 1). Yet this species has not been detected in any of the five saltworks studied to the east of Gibraltar and off the flyway (Fisher exact test, one-tailed  $p = 0.0105$ ).

Ours is the most extensive field demonstration to date that aquatic metazoans can disperse readily via gut passage through birds. Recently, ducks and coots were shown to transport numerous invertebrate eggs of unknown viability (Figuerola et al. 2003). In a previous field demonstration of internal transport, Proctor (1964) found unknown numbers of viable Cladocera and Ostracoda in three ducks. There are increasing numbers of exotic crustaceans and bryozoans observed in aquatic systems (Leppäkoski et al. 2002), and many are likely to have the ability to disperse as resistant eggs via birds (Figuerola and Green 2002a). The conservation of migratory waterbirds is essential to maintain connectivity and indigenous invertebrate biodiversity in the world's wetlands (Amezaga et al. 2002). Nevertheless, their capacity to disperse invasive species within and between continents makes the need to control the importation and release of exotic species at a global level all the more urgent.

There is evidence of competitive exclusion of native *Artemia* by an exotic species. The data available suggest that once *A. franciscana* is detected among native *Artemia* in existing populations, native *Artemia* disappear within a few years (Amat et al. 2005). *A. franciscana* outcompetes *A. parthenogenetica* and *A. salina* within two or three generations under laboratory conditions using few individuals (Abatzopoulos et al. 2002). We expect viable *A. franciscana* propagules to eventually reach all coastal saltworks in the Mediterranean region via birds, but there may be conditions under

which invasion can be resisted, similar to that of other zooplankton communities (Havel and Shurin 2004). Preventing the expansion of aquaculture into protected coastal areas still holding native *Artemia* populations (see Abatzopoulos et al. 2002 for inventory) is likely to be the most effective measure to facilitate their conservation in the short term.

Limnological effects of birds are extremely complex (Daborn et al. 1993). In their seminal paper on ornitholimnology, Hurlbert and Chang (1983) showed how waterbirds may affect the dynamics of invertebrate populations and, indirectly, of entire aquatic ecosystems via predation effects. We suggest they may also do so via dispersal effects.

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